

**PROGRAM ELEMENT 4**  
**Biogeochemical Dynamics**

# Microbiological Controls on the Fate and Transport of Chelated Radionuclides: Multiscale Investigations in Unsaturated Structured Media

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The overall goal of this project is to provide an improved understanding and predictive capability of the mechanisms that allow chelate-degrading bacteria to be effective in the bioremediation of subsurface environments contaminated with toxic metals and radionuclides. The study is motivated by the likelihood that vadose zone microbial activity can effectively consume chelating organic ligands, thus facilitating the immobilization of released metals and radionuclides via sorption and precipitation reactions. The central hypothesis of this study is: in structured soils and aquifers, the biodegradation of metal-chelating ligands is limited by the diffusive mass flux of contaminants from pores too small to be accessed by bacteria to larger pores where bacteria reside.

Our objectives are to (1) develop an improved understanding and predictive capability of the mechanisms governing the biodegradation of cobalt (Co)-NTA in unsaturated, structured media; (2) quantify the microbial and hydrologic conditions that influence the biodegradation of metal-chelating ligands for the purpose of contaminant containment and remediation in heterogeneous, structured media; and (3) provide integrated experimental and theoretical methodologies for the scale-up of biogeochemical processes from the microscopic scale to the macroscale (pedon) scale. Our approach involves the use of a variably saturated dynamic flow technique to quantify the biodegradation of Co<sup>II</sup>NTA as a function of pore class size in structured media and existing models that couple microbial and hydrogeochemical processes to conduct multiscale process and parameter upscaling studies. The experimental results will provide new insights concerning the relationship between the biodegradation of radionuclide-chelate complexes and the pore structure and hydrologic connectivity in heterogeneous subsurface environments. Further, these results will enhance our ability to upscale laboratory- and pedon-scale biodegradation processes to the field scale.

Our results to date demonstrate some of the complex biogeochemistry that influences the fate and transport of chelated metals and radionuclides. The interaction of Co<sup>II</sup>NTA with common soil minerals results in the rapid formation of a variety of highly stable oxidized products, including Co<sup>III</sup>NTA, (Co<sup>III</sup>NTA)<sub>2</sub>, and two isomers of Co<sup>III</sup>(IDA)<sub>2</sub>. These products have been observed in batch systems and in column displacement studies using undisturbed cores of structured saprolite. The stoichiometry of reaction products is a function of soil properties (e.g., pH). There is no direct evidence for the formation of geochemical dissociation products such as Fe<sup>III</sup>NTA or Al<sup>III</sup>NTA. Furthermore, Co(III) complexes exhibit a low affinity for the solid phase. Accordingly, Co injected into undisturbed cores (as Co<sup>II</sup>NTA) exhibits more rapid transport with increasing pH, indicating that the mobile cobalt remains as an anionic ligand-metal complex during transport through the column. Bacterial strains that can degrade NTA and some metal-NTA complexes (e.g., Co<sup>II</sup>NTA) cannot degrade NTA complexed with Co(III). This effect is presumably the result of the kinetic and thermodynamic stability of Co<sup>III</sup>NTA. These observations illustrate some of the complex biogeochemistry that influence the fate and transport of chelated metals and radionuclides. These observed oxidation reactions have far-reaching implications for the transport of Co-NTA in soils and groundwater. The Co(III) complexes have a lower affinity for the solid phase than the corresponding Co(II) complexes resulting in enhanced transport of cobalt. The Co(III) complexes are much more stable, effectively competing with geochemical dissociation reactions and the concomitant formation of surface reactive Co(II). The Co(III) complexes are resistant to biodegradation.

# Complimentary and Inhibitory Coupling of Biological and Geochemical Processes in Metal and Radionuclide Reduction

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Reductive processes, whether abiotic or biotic, may serve to stabilize toxic heavy metals and radionuclides such as chromium and uranium. Direct enzymatic reduction of chromate and uranyl during bacterial respiration is highly desirable as it should lead to reduced products having limited solubilities. Similarly, reduction of  $\text{Co(III)EDTA}^-$  to  $\text{Co(II)EDTA}^{2-}$  serves to diminish the transport of cobalt. Competing electron acceptors present in natural environments may impede or enhance the reduction of a target phase; metabolic products formed during the reduction of alternate electron acceptors may in some cases lead to an enhanced chemical pathway for the reduction of the target element. Here we discuss and demonstrate the influences of competing electron acceptors on the reduction of uranyl, chromate and cobalt(III)-EDTA<sup>-</sup> by dissimilatory iron (DIRB) or sulfur reducing (SRB) bacteria.

Ferric iron is ubiquitous in nature; in many suboxic soils, sediments and subsurface environments it is likely to be the most abundant terminal electron acceptor for microbial respiration. We investigated the microbially mediated, indirect reduction of chromate by *Shewanella alga* strain BrY by injecting a continuous stream of chromate into a suspension of BrY and hydrous ferric oxide minerals. In such systems, Fe(II) produced as a result of microbial respiration serves as a catalyst, reducing Cr(VI) and thus being reoxidized; the production of Fe(II) also provides a means for bacterial tolerance of chromate—an important factor for DIRB sensitive to chromate such as BrY. The prevalence of DIRB implies that chromate reduction may proceed through bacterially mediated pathways in any site containing iron and having limited molecular oxygen. Specific rates for various ferric (hydr)oxides thus provide a means to estimate the extent to which reduction will take place given an influx rate and residence time of chromate contaminated waters.

Similarly, sulfate reducing bacteria generating dissolved sulfide may serve to catalyze the reduction of  $\text{Co(III)EDTA}^-$ . We observed that dissolved sulfide in fact leads to the rapid reduction of  $\text{Co(III)EDTA}^-$  through the formation of highly reactive polysulfide intermediates. Furthermore, in excess sulfide a solid CoS phase is produced. Thus SRB may directly or indirectly lead to the reduction of Co(III) and produce a cobalt phase having limited solubility.

In contrast, ferric iron may potentially serve to restrict the enzymatic reduction of uranyl. Microbial dissimilatory reduction of the highly soluble uranyl ion ( $\text{U}^{\text{VI}}$ ) to relatively insoluble uraninite ( $\text{UO}_2$ ) offers the potential for in situ stabilization. Uranium stabilization by this process is dependent on uranyl being used as the terminal electron acceptor (TEA) in microbial respiration. We examined the reduction of uranyl by BrY in the presence (and absence) of iron hydrous oxides. When cell suspensions of BrY were added to uranyl acetate, uranyl was rapidly removed from solution. Similarly, uranyl adsorbed on goethite underwent dramatic reduction with active BrY cells. In contrast, however, limited reduction was noted when ferrihydrite or amorphous ferric hydroxides were present in suspension. Our results demonstrate that alternate or competing electron acceptors present in soils may modify the reduction of toxic elements; they may either promote or retard the reduction of the target element. In either case, one must consider the specific site geochemistry when evaluating the potential for in-situ reduction of uranyl or chromate.

# Influence of Microbial Nitrate Reduction on Subsurface Iron Biogeochemistry and Contaminant Metal Mobilization

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Our objectives are to determine (1) the extent to which nitrate will inhibit microbial reduction of iron minerals; (2) the effect of mineral reduction on the mobility of representative toxic metals; and (3) the kinetics of nitrate-dependent, microbial oxidation of Fe(II). In the past year, we have examined (i) the effects of  $\text{NO}_3^-$  on microbial reduction of synthetic iron oxides, and the effect of iron reduction on the mobility of zinc which had previously been sorbed to the oxides; (ii) the effects of goethite on  $\text{NO}_3^-$  and  $\text{NO}_2^-$  reduction; (iii) competitive interactions between nitrate and Fe(III) oxide reduction in enrichment cultures of natural sediment bacteria; (iv) microbially-catalyzed nitrate-dependent oxidation of solid-phase Fe(II) compounds at circumneutral pH; and (v)  $\text{Zn}^{2+}$  sorption properties of Fe(III) oxides generated via biological nitrate-dependent oxidation of aqueous Fe(II).

In batch experiments in an artificial groundwater medium containing 10 mM lactate, we previously showed that production of Fe(II) via microbial reduction of synthetic goethite and lepidocrocite is inhibited by the presence of  $\text{NO}_3^-$  and  $\text{NO}_2^-$ . When Zn was adsorbed onto the surface of the goethite or lepidocrocite prior to inoculation with *S. putrefaciens*, the weakly bound Zn (soluble in cold, 0.5 N HCl) was sequestered within a strongly bound phase (soluble in 6 N HCl, but insoluble in 0.5 N HCl). Zn immobilization was not observed in cultures containing  $\text{NO}_3^-$  or  $\text{NO}_2^-$  in which  $\text{Fe}^{2+}$  production was inhibited. Current work is in progress to identify the biogenic minerals responsible for Zn incorporation, to determine if metals other than Zn behave similarly, and to ascertain if similar processes take place during reduction of natural sediments.

Recent work with slurries containing both  $\text{NO}_3^-$  and goethite have demonstrated that the presence of goethite also inhibited  $\text{NO}_3^-$  and  $\text{NO}_2^-$  reduction. Based upon these results and the enhanced production of  $\text{N}_2\text{O}$  in the presence of goethite, we hypothesize that microbially generated  $\text{NO}_2^-$  and Fe(II) abiotically react to form Fe(III) and  $\text{N}_2\text{O}$ . The Fe(III) oxides precipitating on the cell surface can then potentially inhibit transport of  $\text{NO}_2^-$  within the cell. Experiments utilizing differences in  $^{15}\text{N}_2\text{O}$  to discriminate between biogenic  $\text{N}_2\text{O}$  and chemically generated  $\text{N}_2\text{O}$  are underway.

Studies with an autotrophic, circumneutral Fe(II)-oxidizing enrichment culture have demonstrated the potential for rapid, biologically-catalyzed, nitrate-dependent oxidation of solid-phase Fe(II) compounds generated via microbial reduction of amorphous Fe(III) oxide, goethite and two Fe(III) oxide-rich soils. Only very low concentrations (~ 15  $\mu\text{M}$ ) of nitrite were detected in most cultures, suggesting that Fe(II) oxidation was the result of direct enzymatic catalysis rather than abiotic oxidation of Fe(II) coupled to reduction of nitrite generated from the partial reduction of nitrate. Biological nitrate-dependent Fe(II) oxidation has the potential to generate large quantities of reactive Fe(III) oxide surfaces under anaerobic conditions, a phenomenon which has broad-ranging implications for the mobility of metals in saturated subsurface sediments.

We have also examined sorption of  $\text{Zn}^{2+}$  onto Fe(III) oxides generated via microbially-catalyzed nitrate-dependent oxidation of soluble Fe(II) in culture medium containing three different levels of inorganic phosphate (0.05, 0.5 or 1.5 mM). The presence of increasing levels of P led to formation of Fe(III) oxides which were more susceptible to dissolution in dilute (0.5M) HCl during short-term (1 hr) extractions. XRD studies and specific surface area measurements showed that the oxides formed under the different P conditions were all essentially amorphous and had similar specific surface areas (ca. 250  $\text{m}^2/\text{g}$ ). In addition, the different oxides had comparable  $\text{Zn}^{2+}$  sorption properties which were also similar to those determined for high-surface-area goethite preparations.

## The Immobilization of Radionuclides and Metals in the Subsurface by Sulfate-Reducing Bacteria

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This research is designed to evaluate biotechnological approaches to remediating subterranean environments contaminated with radionuclides and heavy metals. Our work specifically focuses on the ability of sulfate-reducing bacterial communities to immobilize U and heavy metal co-contaminants that may be present at selected DOE study sites. Experiments have been designed to (1) elucidate the mechanisms that anaerobes employ to immobilize these materials by reaction with biologically produced sulfide, and (2) evaluate the radionuclides or heavy metals as terminal electron acceptors for subsurface microorganisms. In either eventuality, the environmental risk associated with contaminant migration will be mitigated as the materials will be immobilized and/or precipitated and not easily transported in the subsurface.

This project will involve the pursuit of several objectives, including an evaluation of: (1) the factors influencing desirable immobilization bioconversions (i.e., both natural and co-contaminating electron donors and acceptors, and other site specific variables); (2) the spatial heterogeneity associated with the requisite microbial populations in the subsurface; and (3) the potential for the anaerobic reoxidation and consequent remobilization of the reduced products of the bioconversions.

Groundwater, alluvial floodplain sediments and Mancos shale samples were collected from the UMTRA site at Shiprock, N.M. The goal for this phase of the study was to determine which microbial activities were present in subsurface sediments and groundwater, and whether sulfate reduction activity could be stimulated with the ultimate goal of designing a bioremediation strategy based on the activity of sulfate reducing bacteria.

Populations of microorganisms were enumerated in sediment samples. Significant numbers of methanogens ( $3 \times 10^5$ ), sulfate- ( $3 \times 10^5$ ) and nitrate-reducing ( $2 \times 10^5$ ) bacteria were observed. Smaller numbers of acetogens ( $1 \times 10^4$ ), sulfide-oxidizing ( $2 \times 10^3$ ), Fe(III)-reducing ( $4 \times 10^1$ ), and anaerobic nitrate-dependent Fe(II) oxidizing ( $4 \times 10^2$ ) microorganisms were also present. Time course studies were carried out on sediment samples. Sediments (20 g) were incubated in serum bottles containing 30 ml of filter-sterilized anoxic groundwater under an atmosphere of  $N_2/CO_2$ . Nitrate was rapidly exhausted (within 4 days) in the incubations. After approximately 15 days, sulfate reduction became apparent based on a blackening of the sediments and a decrease in the dissolved sulfate concentration. Sulfate reduction continued over the course of the incubation, indicating that sulfate reduction and consequent metal immobilization was possible in these sediments.

A series of experiments was also performed analyzing for the effect of the presence of clay on sulfate reduction activity in sediments. A general inhibition effect was observed using source clays and commercial clays. Unwashed (but hydrated) clays displayed the most inhibition (4-fold to 65-fold), while washed clays were reduced (1.2-fold to 7.25-fold) in their effectiveness. The most inhibitory included barasym (40-fold) and bentonite (65-fold). Kaolin had a similar effect whether unwashed or washed (10.4 vs. 7.25-fold).

# Biogeochemistry of Technetium: Effects of Enzymatic Metal Reduction on Speciation and Potential for Transport and Remediation in Porous Subsurface Media

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Development of remedial measures for technetium (Tc) and other contaminants that are transported in aerobic groundwaters as oxyanions will require a fundamental understanding of the mechanisms of microbial and abiotic reduction and the factors controlling the effects of these processes on form and mobility. The effect of electron donor on the rate and extent of pertechnetate ion [Tc(VII)O<sub>4</sub>] reduction by the subsurface metal-reducing bacterium *Shewanella putrefaciens* CN32 was determined, and the aqueous and solid phase reduction products formed in the presence and absence of inorganic and organic complexing ligands (bicarbonate, DTPA, EDTA, citrate) were evaluated through a combination of high resolution transmission electron microscopy, X-ray absorption spectroscopy and thermodynamic calculations. The kinetics of these reactions were contrasted to Tc(VII) reduction by Fe(II)<sub>aq</sub> and Fe(II) sorbed on goethite.

When H<sub>2</sub> served as the electron donor, dissolved Tc(VII) was rapidly reduced to amorphous Tc(IV) hydrous oxide, which was largely associated with the cell periplasm and the outer cell surface in unbuffered 0.85% NaCl and with extracellular particulates (0.2 – 0.001 μm) in bicarbonate buffer. The reduction rate was much lower when lactate was the electron donor, with extracellular Tc(IV) hydrous oxide also the dominant solid-phase reduction product. In bicarbonate systems, soluble (< 0.001 μm) electronegative Tc(IV) carbonate complexes were also formed that exceeded Tc(VII) in electrophoretic mobility. Thermodynamic calculations indicated that NaCl solutions without complexing ligands were oversaturated with respect to Tc(IV) hydrous oxide which would be present from pH 4 to 9 and at Eh values <50 mV, and that negatively-charged aqueous Tc(IV) carbonate species would dominate in carbonate solutions from pH 5.5 to 10.5 and at Eh values <200 mV. Thus, carbonate complexes may represent an important pathway for Tc transport in anaerobic subsurface environments where it has generally been assumed that Tc mobility is controlled by low solubility Tc(IV) hydrous oxide and adsorptive, aqueous Tc(IV) hydrolysis products. The presence of organic complexing ligands during microbial reduction of Tc(VII) in NaCl resulted in the formation of aqueous complexes of Tc(IV,V) and investigations are underway to further define their chemical speciation and potential for transport in groundwaters.

In abiotic experiments, Tc(VII) was reduced slowly in the presence of Fe(II) in water, but was rapidly reduced to Tc(IV) by Fe(II) sorbed on goethite. At higher sorbed Fe(II) concentrations (near saturation), the rate of Tc(VII) reduction exceeded direct microbial reduction even with H<sub>2</sub> as the electron donor, suggesting that sorbed Fe(II) resulting from biogenic processes may be an important reducing agent for Tc(VII) in groundwater systems. The effective redox potential and kinetics of electron transfer for biogenic Fe(II) will depend specifically on the chemical/mineralogic environment in which the Fe(II) resides. Investigations are underway to determine the relative importance of direct and indirect reductive processes for Tc(VII) in static and advective experimental systems.

## Solubilization of Radionuclides and Metals by Iron Reducing Bacteria

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Iron oxides are ubiquitous mineralogic components of vadose zone and aquifer sediments, typically existing as particle coatings and intergrain cements. Fe(III) oxides are reactive, act to buffer subsurface redox capacity, and are strong sorbents of metallic and radionuclide contaminants. Iron oxides serve as a major in-ground repository of contaminants on DOE lands. Ascertaining the long term stability of contaminant-Fe(III) oxide associations and devising means to effectively extract sorbed contaminants are important scientific needs for DOE site remediation and closure that are being investigated by this project.

Research is investigating hydrogeochemical controls on the activity of dissimilatory iron-reducing bacteria (DIRB) in subsurface sediments and the potential of DIRB for solubilizing sorbed metals associated with Fe(III) oxides under anoxic conditions. The project focuses on bacterial ferrous iron mineralization and the impact of biomineralization on redox buffering capacity and contaminant solubility. Batch and stirred-flow reactors with synthetic and subsurface materials are inoculated with DIRB to investigate how different C-sources and e-donors, interfacial chemical reactions and water advection meter the activity of DIRB. The reductive solubilization of sorbed metals by DIRB is investigated using synthetic and subsurface materials spiked and aged with important DOE contaminants [Ni(II), Hg(II), Co(II/III) and Eu(III)], and a contaminated subsurface material. A novel biogeochemical modeling approach is used for experiment interpretation whereby surface and aqueous complexation reactions and precipitation are linked with Monod kinetics and water transport. Mossbauer spectroscopy, X-ray adsorption spectroscopy and other techniques are used to characterize the chemical and mineralogic nature of the Fe(III) oxides, biomineralization products and biogenic contaminant host phases as a basis for chemical model development.

In the first project year we have shown that: (1) hematite and goethite in subsurface sediments are more bioavailable (to DIRB) than synthetic goethite and hematite because of crystallite disorder and surface heterogeneities, and (2) Co(III) and Ni(II) can be solubilized from crystalline Fe(III) oxides by DIRB in mole fraction excess to iron leading to their net depletion from the oxide phase. In the second year, we have investigated the bioreduction of fresh, aged and heated hydrous ferric oxide (HFO) spiked with Ni and Co. Aging and heating produces a poly-phase Fe(III) oxide association much like those found in subsurface materials. Using a combination of Mossbauer spectroscopy and high resolution electron microscopies, we have found that sorbed contaminants inhibit the crystallization of HFO, alter bioreduction through a combination of physiologic and surface chemical effects, and direct the formation of different biomineralization suites, some of which are highly effective at contaminant capture through coprecipitation. We have determined biogeochemical conditions favoring both mobilization and immobilization of contaminants and are now linking geochemical and microbiologic modeling to quantitatively describe these processes.